Exhibit C

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Carbohydrate diversity: synthesis of glycoconjugates and complex carbohydrates

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The fundamental role of glycoconjugates in many biological processes is now well appreciated and has intensified the development of innovative and improved synthetic strategies. All areas of synthetic methodology have seen major advances and many complex, highly branched carbohydrates and glycoproteins have been prepared using solution- and/or solid-phase approaches. The development of an automated oligosaccharide synthesizer provides rapid access to biologically relevant compounds. These chemical approaches help to produce sufficient quantities of defined oligosaccharides for biological studies. Synthetic chemistry also supports an improved understanding of glycobiology and will eventually result in the discovery of new therapeutics.

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Abbreviations

GlcNAc	*N*-acetylglucosamine
GPI	glycosylphosphatidylinositol
HIV	human immunodeficiency virus
PSA	prostate-specific antigen

Introduction

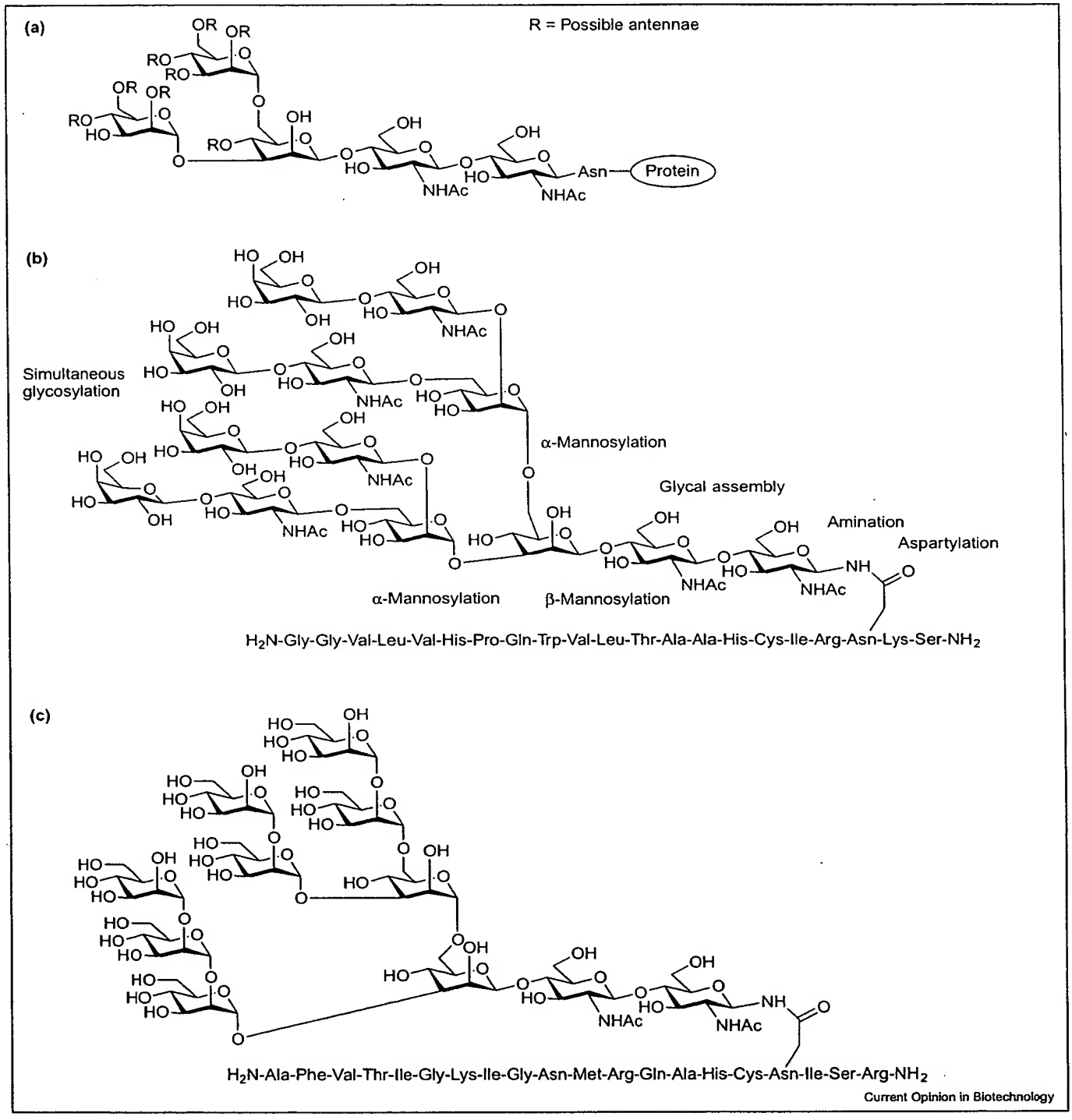
In addition to oligopeptides and oligonucleotides, oligosaccharides (glycans) constitute the third major class of naturally occurring biopolymers that play a fundamental role in many important biological processes. Glycans are commonly found in nature as glycoconjugates (glycoproteins or glycolipids) that show high structural diversity, greatly exceeding the diversity of proteins and nucleic acids.

In contrast to linear oligopeptides and oligonucleotides, oligosaccharides are often complex branched molecules and the glycan core is commonly attached to proteins and lipids. In nature, three major classes of glycans exist: *N*-linked glycans, *O*-linked glycans and glycosylphosphatidylinositol (GPI) anchors. Intensive research into the biological role of carbohydrates has led to an increased need for sufficient quantities of natural and modified glycoproteins; however, the isolation of carbohydrates from natural sources is extremely difficult owing to their structural complexity. Access to pure carbohydrates for biological, biochemical, biophysical and medicinal studies therefore relies on chemical and enzymatic synthesis [1,2]. Remarkable progress has been made in this area; however, further innovations are required to handle the structural complexity of oligosaccharides. Their preparation is technically difficult, extremely time-consuming and performed by a few specialized laboratories. The introduction of solid-phase synthesis strategies has significantly improved carbohydrate assembly, as an excess of reagent can be used to ensure high yields and to reduce the number of purification steps. The development of an automated oligosaccharide synthesizer [3•,4•,5••] has led to rapid access to complex carbohydrates of biological relevance. This review highlights recent advances in the synthesis of complex oligosaccharides and glycoproteins, primarily focusing on strategies published in the past two years.

N-Linked glycoproteins

N-Linked glycoproteins (*N*-glycans) are the most abundant in nature and are commonly divided into four groups: high-mannose, complex, hybrid and poly-*N*-acetyllactosamine glycans. Although the structural details are well established, little is known about their structure–activity relationship. In *N*-glycans, the oligosaccharide sidechain is attached to the protein via an asparagine amino acid. All *N*-glycans share the common pentasaccharide core structure (mannose)$_3$(*N*-acetylglucosamine)$_2$ ($Man_3GlcNAc_2$) shown in Figure 1a. Structural diversity is generated by variation in the substitution pattern of the pentasaccharide core, in the degree of branching and in the terminal sugars. The pentasaccharide core can be extended by up to five antennae. The preparation of the basic structure contains several synthetic challenges, including branching and the inclusion of a β-mannoside. Recently, two efficient partial syntheses of the core structure have been accomplished [6,7], selectively establishing the β-mannosidic linkage. The orthogonally protected β-mannosylated chitobiose trisaccharide with a terminal azido group serves as a key building block in the preparation of complex *N*-glycans. The entire pentasaccharide has been synthesized recently by Danishefsky and colleagues [8]

Figure 1



N-Linked glycoproteins. **(a)** Structure of the core pentasaccharide common to all *N*-glycans. The core structure can be extended by up to five antennae (R). **(b)** Structure of a prostate-specific antigen (PSA) glycopeptide. The crucial retrosynthetic steps of Danishefsky's [21•] strategy are shown. **(c)** Structure of the gp120 glycopeptide fragment, which is a possible target for an anti-HIV-vaccine. Protein sequences are shown using the three-letter amino acid code.

using Crich's β-mannosylation methodology [6] followed by a simultaneous di-α-mannosylation with a thiomannoside donor.

As an alternative to these solution-phase preparations, the synthesis of the core pentasaccharide selectively functionalized with one *N*-acetylglucosamine residue has been performed recently using a solid-phase approach [9]. The first automated solid-phase oligosaccharide synthesizer [5••] has been used to efficiently prepare the core pentasaccharide [10] by using an octenediol functionalized Merrifield's resin and three different building blocks: two monosaccharides and one disaccharide already containing the β-mannosidic linkage. Branching was achieved by simultaneous dimannosylation of the trisaccharide core.

Innovative synthetic methods have also provided access to more complex and highly branched *N*-glycans. Weiss and Unverzagt [11] have developed a general strategy for the preparation of multiantennary *N*-glycans. Crucial challenges in the synthesis of these sterically crowded bi- to tetra-antennary compounds is the sequence of introducing the building blocks and the steric demand of the building blocks. Complex biantennary *N*-glycans are also accessible via chemoenzymatic total synthesis. Elongation of synthetic oligosaccharides has been performed using glycosyltransferases to give full-length *N*-glycans [12,13].

Synthetic oligosaccharides are useful in gaining a more detailed understanding of glycoprotein quality control. In particular, maintenance of the integrity of protein folding has recently received significant attention. Ito and colleagues [14,15] accomplished a convergent and stereoselective route to the nonasaccharide $Man_8GlcNAc_2$ and the monoglucosylated dodecasaccharide α-$Glc_1Man_9GlcNAc_2$, a putative ligand of the molecular chaperones calnexin and calreticurin. These synthetic oligosaccharides might serve as molecular probes to detect glycoprotein–mannosidase-like protein recognition.

Glycoproteins are also important in the context of diagnostics, therapeutics and vaccines. The integration of oligosaccharides into glycoproteins is realized by converting them into anomeric glycosylamines, which is either performed by treatment with ammonium hydrogencarbonate or by reduction of anomeric glycosyl azides, and subsequent attachment to the peptide chain [16–18]. Guo and colleagues [19] attached a fucoslyated trisaccharide to the peptide of the CD52 antigen by using a solution-phase synthesis with solid-phase workup or a combined solution- and solid-phase approach. More complex oligosaccharides containing two thiol residues were linked to the same peptide by 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide synthesis [20]. Because of their short peptide chain containing only 12 amino acids, their simple glycosylation pattern and their interesting bioactivity, these glycopeptides serve as useful models to study structure–activity relationships. The development of a universal strategy [21•] for the preparation of complex multibranched *N*-acetyllactosamine-type glycans from common precursors has led to the first chemical synthesis of normal and transformed prostate-specific antigen (PSA) glycopeptides (Figure 1b). PSA has been identified as a highly specific cancer marker that might enable the early diagnosis of prostate tumours.

N-Linked carbohydrates also play an important role in human immunodeficiency virus (HIV) retroviral pathogenesis. The HIV-1 surface envelope glycoprotein gp120 is highly glycosylated containing up to 24 *N*-linked high-mannose carbohydrates and shows biological functions in helper T-lymphocyte infections [22]. Seeberger and colleagues [23] developed a linear solution-phase synthesis of a triantennary high-mannose nonasaccharide from gp120 using just three monosaccharide building blocks. Employing a reactivity-based one-pot self-condensation approach, Wong and coworkers [24] prepared several high-mannose oligosaccharides, which efficiently inhibit the binding of the antibody 2G12 to gp120. More recently, Danishefsky and colleagues [25••,26••] described the first chemical synthesis of HIV gp120 fragments (Figure 1c), which serve as targets for an anti-HIV vaccine.

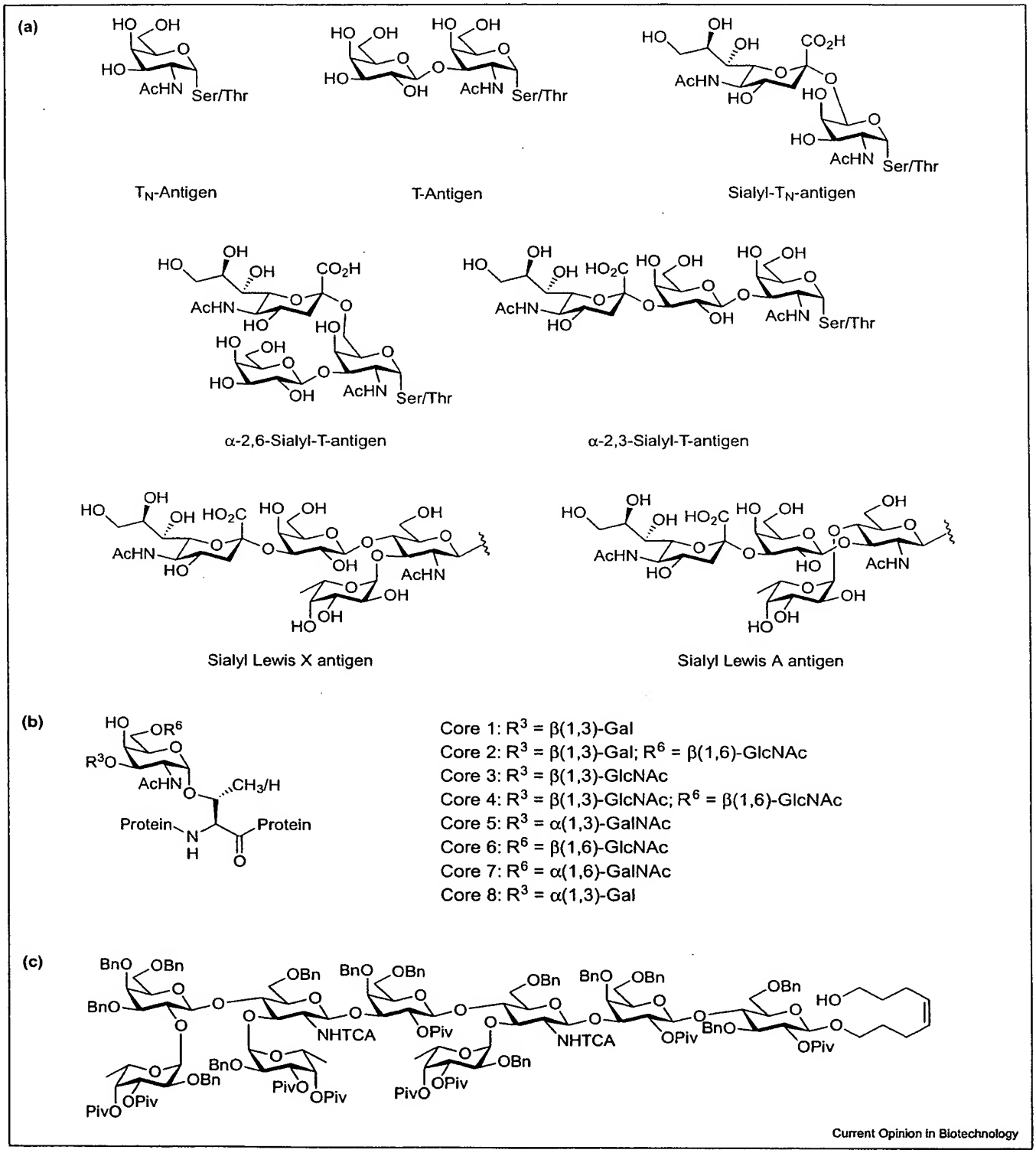
Many important glycoproteins are multiply glycosylated at fixed sites. Danishefsky's laboratory [27] recently disclosed a convergent method for the preparation of bifunctional glycopeptides: two glycopeptides are synthesized separately from their glycan and peptide precursors using standard procedures and subsequently coupled to yield the bifunctional compounds.

O-Linked glycoproteins

A second major group of biologically important glycoproteins are *O*-linked glycoproteins (*O*-glycans). The carbohydrate residue in *O*-glycans is covalently attached to the peptide backbone via the hydroxyl group of serine, threonine, tyrosine, hydroxyproline, hydroxylysine or another hydroxylated amino acid. In contrast to *N*-glycans, these glycoproteins show a higher degree of structural diversity and do not share a common core structure. Additional variety arises from further carbohydrate elongations of these backbones.

Tumour-associated antigens (Figure 2a) like the T_N-, T-, sialyl-T_N and sialyl-T antigens as well as the sialyl Lewis X and sialyl Lewis A antigens were first found in mucins. Mucins are a class of highly *O*-glycosylated proteins present on the surface of various types of epithelial cells. In normal tissue, the peptide backbone carries several complex oligosaccharides derived from the glycan core structures shown in Figure 2b, which are characterized by

Figure 2



O-Linked glycoproteins. **(a)** Structures of tumour-associated carbohydrate antigens that were first discovered in mucins. **(b)** Core structures of mucin-type *O*-linked glycans, a class of highly *O*-glycosylated proteins. **(c)** Structure of the Le^y-Le^x tumour marker. Ac, acetyl; Bn, benzyl; Piv, pivaloyl; TCA, trichloroacetyl.

an *N*-acetylgalactosamine unit α-*O*-linked to serine or threonine. An increased expression of mucins is usually prevalent in tumour cells, where the carbohydrate chains are modified due to incomplete glycosylation and premature sialylation. As tumour-associated glycans with peptide sequences of mucins constitute a promising target for the development of synthetic antitumour vaccines, the chemical synthesis of such glycoconjugates has received considerable attention and several reviews devoted to this field of research have been published [28–30,31•].

A solid-phase approach [32,33] has been used for the stereoselective construction of several different mucin-type *O*-glycans. Stepwise elongation of the carbohydrate led to the required highly glycosylated amino acid building blocks, which were then incorporated into a solid-phase glycopeptide synthesis. Other branched *O*-glycans have recently been prepared by an efficient one-pot glycosylation approach using either glycosyl fluoride [34] or thioglycoside [35] building blocks. As sialylated derivatives of tumour-associated antigens are also present on the surface of cancer cells, the preparation of *O*-linked sialyl oligosaccharides is important. Paulson and colleagues [36] demonstrated that recombinant sialyltransferases are ideal catalysts for the simple and efficient preparation of *O*-linked sialyl oligosaccharides by elongation of a synthetic glycosyl amino acid.

The application of non-natural amino acids in carbohydrate vaccines has also attracted considerable attention [37], as these unnatural linkages might give an increased immune response. Danishefsky's group [38] investigated the synthesis of different glycosyl hydroxynorleucines, each containing a tumour-associated carbohydrate antigen. While the glycosylation of trichloroacetimidate donors with the amino acid predominately afforded the corresponding α-*O*-linked product, the reaction with a glycal epoxide donor provided the β-*O*-linked product. The glycal methodology was also successfully applied to the synthesis of Lewis Y- and Globo-H-containing amino acids.

More recently, an automated synthesizer has been used to accelerate the synthesis of the Lewis^{y}-Lewis^{x} tumour marker (Figure 2c) and the Lewis X and Lewis Y blood group antigens [39••]. Only five monomers were necessary for the efficient construction of the three target structures.

GPI anchors

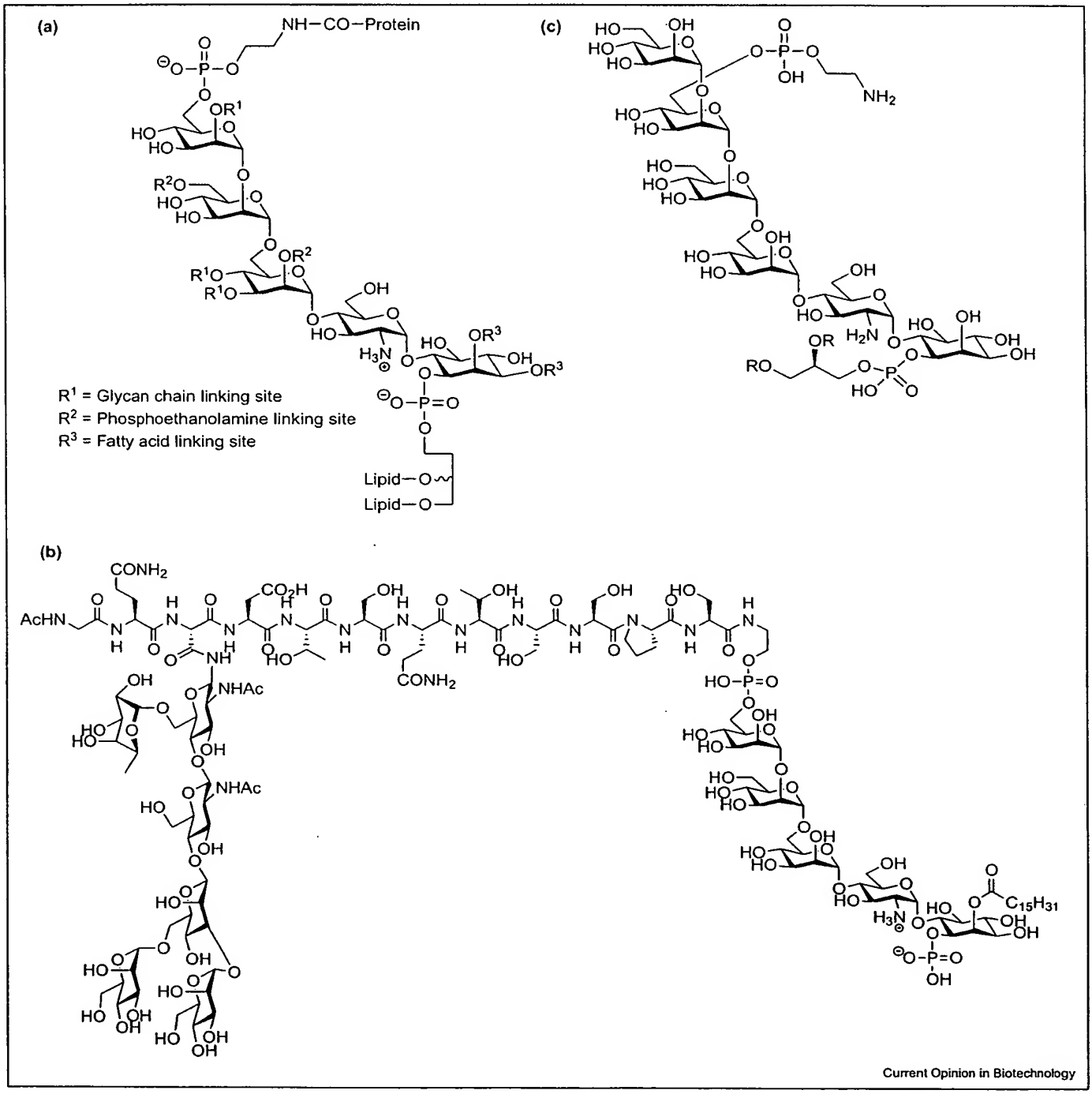
GPI-anchored proteins are involved in many biological and physiological processes and have attracted considerable attention since the first structure determination of a GPI in 1988 [40]. These naturally occurring glycolipids serve to attach proteins or glycoproteins onto eukaryotic cell membranes. All reported GPI structures share the basic core structure shown in Figure 3a with a linear tetrasaccharide attached to the 6-*O*-position of inositol. Besides this conserved general structure, considerable diversity exists within the GPI anchor family based on the variation of the substitution pattern on this pseudo-pentasaccharide backbone. In most cases, the core is further modified by species-specific carbohydrates, additional phosphoethanolamine units and variations in the lipid moiety. Proteins or glycoproteins are linked to the non-reducing end by their C termini or a phosphoethanolamine group. Owing to the structural complexity of the GPI anchors that requires a detailed knowledge of lipid, phosphate and oligosaccharide chemistry, many chemists have focused on the synthesis of these motifs [41•].

A linear solution-phase approach allows for the construction of complex GPI anchors and for the preparation of an orthogonally protected derivative of the phosphorylated pseudo-pentasaccharide core [42]. Another variable concept for the preparation of branched GPIs was developed by Pekari and Schmidt [43]. The efficiency of this approach was demonstrated by the synthesis of the GPI anchors of rat brain Thy-1 and scrapie prion protein in their water-soluble and lipidated forms. This approach also allows further attachment of peptide residues or biological markers to the GPI anchor. Reichardt and Martin-Lomas [44] reported a soluble support-based approach for the synthesis of the GPI backbone. This method, using a polyethylene glycol-grafted polystyrene resin functionalized with a Wang-chloride linker, can be applied to the preparation of a small library of GPI precursors.

CD52 antigens, simple GPI-anchored glycopeptides, are present on eukaryotic cells and play an important role in the human immune system. Initial studies aimed at the synthesis of sperm CD52, including the preparation of an acylated inositol [45] and the linkage to the peptide [46], were performed by Guo and colleagues. More recently, they reported [47••] the first synthesis of a skeleton structure of sperm CD52. In their strategy the glycopeptide and the GPI anchor were prepared separately and subsequently linked by an amide bond to give the glycopeptide–GPI conjugate (Figure 3b).

Synthetic GPIs are promising vaccine candidates against malaria, as shown in a mouse model [48]. Annually, malaria infects 5–10% of the world's population and kills about 3 million people each year. The malaria parasite *Plasmodium falciparum* expresses a large amount of GPI anchored to a protein, and the GPI structure (Figure 3c) has been identified as the malaria toxin. A solution-phase synthesis of two malaria vaccine candidates with a pseudo-hexasaccharide backbone has recently been reported by Seeberger and collagues [49]. This strategy allows for scale-up to procure compounds for preclinical

Figure 3



GPI anchors. **(a)** Basic core structure of all GPI anchors. **(b)** Skeleton structure of sperm CD52. **(c)** Structure of the malaria GPI vaccine candidate.

and clinical trials. The authors also demonstrated that the synthesis of this target can be automated effectively [50••]. The fully protected oligosaccharide was obtained in only 9 h starting from four monosaccharides and one disaccharide building block. Fraser-Reid and coworkers [51,52••] developed a method for the solution-phase

synthesis of a fully lipidated and phosphorylated malarial GPI pseudo-pentasaccharide using orthoesters and methyl α-D-glucopyranoside as the key building blocks.

Conclusions

Innovative synthetic methods are an important tool to create diverse carbohydrates. Recent advances in the preparation of complex oligosaccharides as well as entire glycoproteins containing *N*-glycans, *O*-glycans and GPI anchors have been highlighted in this review. Highly branched carbohydrates and biologically relevant oligosaccharides are now accessible via these methods, providing sufficient quantities for biological studies. The availability of defined synthetic glycoproteins and glycolipids will significantly support biological investigations. The development of new strategies for the preparation of carbohydrates is fundamental for the understanding of carbohydrate–protein interactions, biosynthetic pathways and structure–activity relationships and will allow for the discovery of new targets for therapeutics, diagnostics and vaccines. The introduction of an automated oligosaccharide synthesizer has greatly accelerated access to many highly branched carbohydrates, and a series of biologically relevant oligosaccharides has been efficiently prepared on this machine. Further improvement and extension of this technology could allow for the automated synthesis of complex glycoproteins, proteoglycans and glycolipids using only one instrument and will eventually enable even non-specialists to create biologically important compounds for biochemical, biophysical and medicinal applications.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Davis BG: **Synthesis of glycoproteins.** *Chem Rev* 2002, **102**:579-601.

2. Macmillan D, Daines AM: **Recent developments in the synthesis and discovery of oligosaccharides and glycoconjugates for the treatment of disease.** *Curr Med Chem* 2003, **10**:2733-2773.

3. • Seeberger PH: **Automated carbohydrate synthesis to drive chemical glycomics.** *Chem Commun* 2003:1115-1121.
See annotation for [5••].

4. • Palmacci ER, Plante OJ, Hewitt MC, Seeberger PH: **Automated synthesis of oligosaccharides.** *Helv Chim Acta* 2003, **86**:3975-3990.
See annotation for [5••].

5. •• Plante OJ, Palmacci ER, Seeberger PH: **Automated solid-phase synthesis of oligosaccharides.** *Science* 2001, **291**:1523-1527.
The authors describe the first automated solid-phase oligosaccharide synthesizer for the preparation of linear and branched structures up to dodecasaccharides. New glycosylating agents, linker, coupling cycles and the use of high-resolution magic angle spinning NMR to follow reactions on polymer support are discussed.

6. Dudkin VY, Crich D: **A short synthesis of the trisaccharide building block of the *N*-linked glycans.** *Tetrahedron Lett* 2003, **44**:1787-1789.

7. Unverzagt C: **Synthesis of a core trisaccharide as a versatile building block for *N*-glycans and glycoconjugates.** *Chem Eur J* 2003, **9**:1369-1376.

8. Dudkin VY, Miller JS, Danishefsky SJ: **A concise route to the core pentasaccharide of *N*-linked glycoproteins.** *Tetrahedron Lett* 2003, **44**:1791-1793.

9. Wu XY, Grathwohl M, Schmidt RR: **Efficient solid-phase synthesis of a complex, branched *N*-glycan hexasaccharide: use of a novel linker and temporary-protecting-group pattern.** *Angew Chem Int Edit* 2002, **41**:4489-4493.

10. Ratner DM, Swanson ER, Seeberger PH: **Automated synthesis of a protected *N*-linked glycoprotein core pentasaccharide.** *Org Lett* 2003, **5**:4717-4720.

11. Weiss H, Unverzagt C: **Highly branched oligosaccharides: a general strategy for the synthesis of multiantennary *N*-glycans with a bisected motif.** *Angew Chem Int Edit* 2003, **42**:4261-4263.

12. Unverzagt C, Andre S, Seifert J, Kojima S, Fink C, Srikrishna G, Freeze H, Kayser K, Gabius HJ: **Structure-activity profiles of complex biantennary glycans with core fucosylation and with/without additional α2,3/α2,6 sialylation: synthesis of neoglycoproteins and their properties in lectin assays, cell binding, and organ uptake.** *J Med Chem* 2002, **45**:478-491.

13. Prahl I, Unverzagt C: **Enzymatic elongation of the LEC14 antigen generates a β-1,2 arm on N-glycans.** *Angew Chem Int Edit* 2002, **41**:4259-4262.

14. Matsuo I, Wada M, Manabe S, Yamaguchi Y, Otake K, Kato K, Ito Y: **Synthesis of monoglucosylated high-mannose-type dodecasaccharide, a putative ligand for molecular chaperone, calnexin, and calreticurin.** *J Am Chem Soc* 2003, **125**:3402-3403.

15. Matsuo I, Ito Y: **Synthesis of an octamannosyled glycan chain, the key oligosaccharide structure in ER-associated degradation.** *Carbohydr Res* 2003, **338**:2163-2168.

16. Totani K, Matsuo I, Ito Y: **Tight binding ligand approach to oligosaccharide-grafted protein.** *Bioorg Med Chem Lett* 2004, **14**:2285-2289.

17. Hojo H, Haginoya E, Matsumoto Y, Nakahara Y, Nabeshima K, Toole BP, Watanabe Y: **The first synthesis of peptide thioester carrying *N*-linked core pentasaccharide through modified Fmoc thioester preparation: synthesis of an *N*-glycosylated Ig domain of emmprin.** *Tetrahedron Lett* 2003, **44**:2961-2964.

18. Miller JS, Dudkin VY, Lyon GJ, Muir TW, Danishefsky SJ: **Toward fully synthetic *N*-linked glycoproteins.** *Angew Chem Int Edit* 2003, **42**:431-434.

19. Shao N, Xue J, Guo ZW: **Chemical synthesis of CD52 glycopeptides containing the acid-labile fucosyl linkage.** *J Org Chem* 2003, **68**:9003-9011.

20. Pratt MR, Bertozzi CR: **Chemoselective ligation applied to the synthesis of a biantennary N-linked glycoform of CD52.** *J Am Chem Soc* 2003, **125**:6149-6159.

21. • Dudkin VY, Miller JS, Danishefsky SJ: **Chemical synthesis of normal and transformed PSA glycopeptides.** *J Am Chem Soc* 2004, **126**:736-738.
A universal method for the preparation of N-linked glycans from a common precursor has been established and efficiently applied to the synthesis of normal and transformed PSA fragments.

22. Bewley CA, Gustafson KR, Boyd MR, Covell DG, Bax A, Clore GM, Gronenborn AM: **Solution structure of cyanovirin-N, a potent HIV-inactivating protein.** *Nat Struct Biol* 1998, **5**:571-578.

23. Ratner DM, Plante OJ, Seeberger PH: **A linear synthesis of branched high-mannose oligosaccharides from the HIV-1 viral surface envelope glycoprotein gp120.** *Eur J Org Chem* 2002:826-833.

24. Lee HK, Scanlan CN, Huang CY, Chang AY, Calarese DA, Dwek RA, Rudd PM, Burton DR, Wilson IA, Wong CH: **Reactivity-based one-pot synthesis of oligomannoses: defining antigens recognized by 2G12, a broadly neutralizing anti-HIV-1 antibody.** *Angew Chem Int Edit* 2004, **43**:1000-1003.

25. Mandal M, Dudkin VY, Geng XD, Danishefsky S: **In pursuit of**
•• **carbohydrate-based HIV vaccines, Part 1: The total synthesis of hybrid-type gp120 fragments.** *Angew Chem Int Edit* 2004, **43**:2557-2561.
The first chemical synthesis of mature hybrid type HIV gp120 glycopeptide fragments is reported, which may serve as anti-HIV vaccines.

26. Geng XD, Dudkin VY, Mandal M, Danishefsky SJ: **In pursuit of**
•• **carbohydrate-based HIV vaccines, Part 2: The total synthesis of high-mannose-type gp120 fragments-evaluation of strategies directed to maximal convergence.** *Angew Chem Int Edit* 2004, **43**:2562-2565.
The first chemical synthesis of high-mannose type HIV gp120 glycopeptide fragments has been achieved using either a 'layered' or a 'block' assembly oligosaccharide approach. The glycans were then linked to gp120 peptide fragments by direct aspartylation.

27. Warren JD, Miller JS, Keding SJ, Danishefsky SJ: **Toward fully synthetic glycoproteins by ultimately convergent routes: a solution to a long-standing problem.** *J Am Chem Soc* 2004, **126**:6576-6578.

28. Dziadek S, Espinola CG, Kunz H: **Synthetic glycopeptides for the development of antitumour vaccines.** *Aust J Chem* 2003, **56**:519-543.

29. Dziadek S, Kunz H: **Synthesis of tumor-associated glycopeptide antigens for the development of tumor-selective vaccines.** *Chem Rec* 2004, **3**:308-321.

30. Marcaurelle LA, Bertozzi CR: **Recent advances in the chemical synthesis of mucin-like glycoproteins.** *Glycobiology* 2002, **12**:69R-77R.

31. Brocke C, Kunz H: **Synthesis of tumor-associated glycopeptide**
• **antigens.** *Bioorg Med Chem* 2002, **10**:3085-3112.
A good review summarizing the synthesis of tumour-associated glycopeptide antigens.

32. Takano Y, Habiro M, Someya M, Hojo H, Nakahara Y: **Preparation of core 2 type tetrasaccharide carrying decapeptide by benzyl protection-based solid-phase synthesis strategy.** *Tetrahedron Lett* 2002, **43**:8395-8399.

33. Brocke C, Kunz H: **Synthetic tumor-associated glycopeptide antigens from the tandem repeat sequence of the epithelial mucin MUC4.** *Synthesis* 2004:525-542.

34. Tanaka H, Adachi M, Takahashi T: **Efficient synthesis of core 2 class glycosyl amino acids by one-pot glycosylation approach.** *Tetrahedron Lett* 2004, **45**:1433-1436.

35. Hashihayata T, Ikegai K, Takeuchi K, Jona H, Mukaiyama T: **Convergent total syntheses of oligosaccharides by one-pot sequential stereoselective glycosylations.** *Bull Chem Soc Jpn* 2003, **76**:1829-1848.

36. Blixt O, Allin K, Pereira L, Datta A, Paulson JC: **Efficient chemoenzymatic synthesis of O-linked sialyl oligosaccharides.** *J Am Chem Soc* 2002, **124**:5739-5746.

37. Allen JR, Harris CR, Danishefsky SJ: **Pursuit of optimal carbohydrate-based anticancer vaccines: preparation of a multiantigenic unimolecular glycopeptide containing the Tn, MBr1, and Lewis(y) antigens.** *J Am Chem Soc* 2001, **123**:1890-1897.

38. Keding SJ, Endo A, Danishefsky SJ: **Synthesis of non-natural glycosylamino acids containing tumor-associated carbohydrate antigens.** *Tetrahedron* 2003, **59**:7023-7031.

39. Love KR, Seeberger PH: **Automated solid-phase synthesis of**
•• **protected tumor-associated antigen and blood group determinant oligosaccharides.** *Angew Chem Int Edit* 2004, **43**:602-605.
The synthesis of the Le^y-Le^x nonasaccharide, the Lewis X pentasaccharide and the Lewis Y hexasaccharide was efficiently performed on an automated synthesizer. Only five monomeric building blocks were necessary for the assembly of these target structures and the synthesis of the nonasaccharide was completed within 23 h.

40. Ferguson MAJ, Williams AF: **Cell-surface anchoring of proteins via glycosyl- phosphatidylinositol structures.** *Annu Rev Biochem* 1988, **57**:285-320.

41. Guo ZW, Bishop L: **Chemical synthesis of GPIs and**
• **GPI-anchored glycopeptides.** *Eur J Org Chem* 2004:3585-3596.
An excellent review summarizing recent approaches for the chemical synthesis of GPI anchors.

42. Lahmann M, Garegg PJ, Konradsson P, Oscarson S: **Synthesis of a polyphosphorylated GPI-anchor core structure.** *Can J Chem* 2002, **80**:1105-1111.

43. Pekari K, Schmidt RR: **A variable concept for the preparation of branched glycosyl phosphatidyl inositol anchors.** *J Org Chem* 2003, **68**:1295-1308.

44. Reichardt NC, Martin-Lomas M: **A practical solid-phase synthesis of glycosylphosphatidylinositol precursors.** *Angew Chem Int Edit* 2003, **42**:4674-4677.

45. Xue J, Guo ZW: **Convergent synthesis of a GPI containing an acylated inositol.** *J Am Chem Soc* 2003, **125**:16334-16339.

46. Xue J, Shao N, Guo ZW: **First total synthesis of a GPI-anchored peptide.** *J Org Chem* 2003, **68**:4020-4029.

47. Shao N, Xue B, Guo ZW: **Chemical synthesis of a skeleton**
•• **structure of sperm CD52 — a GPI-anchored glycopeptide.** *Angew Chem Int Edit* 2004, **43**:1569-1573.
The first report of the chemical synthesis of a complex and all natively linked glycopeptide–GPI conjugate. The preparation of a skeleton structure of the sperm CD52 antigen was achieved by first carrying out the separate synthesis of the protected glycopeptides and the GPI. After selective deprotection both segments were coupled by an amide bond to give the entire conjugate.

48. Schofield L, Hewitt MC, Evans K, Siomos MA, Seeberger PH: **Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria.** *Nature* 2002, **418**:785-789.

49. Seeberger PH, Soucy RL, Kwon Y-U, Snyder DA, Kanemitsu T: **A convergent, versatile route to two synthetic conjugate anti-toxin malaria vaccines.** *Chem Commun* 2004:1706-1707.

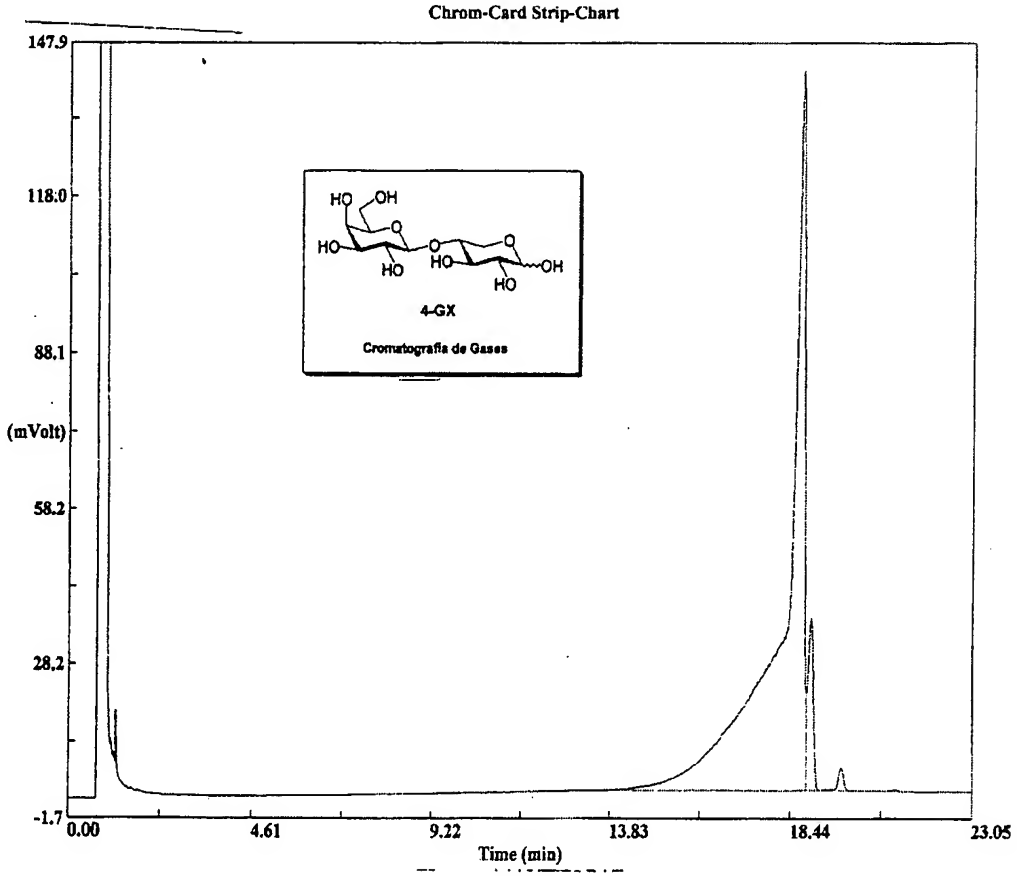
50. Hewitt MC, Snyder DA, Seeberger PH: **Rapid synthesis of a**
•• **glycosylphosphatidylinositol-based malaria vaccine using automated solid-phase oligosaccharide synthesis.** *J Am Chem Soc* 2002, **124**:13434-13436.
The automated synthesis of a hexasaccharide, which constitutes a promising malaria vaccine, has been carried out.

51. Lu J, Jayaprakash KN, Fraser-Reid B: **First synthesis of a malarial prototype: a fully lipidated and phosphorylated GPI membrane anchor.** *Tetrahedron Lett* 2004, **45**:879-882.

52. Lu J, Jayaprakash KN, Schlueter U, Fraser-Reid B: **Synthesis**
•• **of a malaria candidate glycosylphosphatidylinositol (GPI) structure: a strategy for fully inositol acylated and phosphorylated GPIs.** *J Am Chem Soc* 2004, **126**:7540-7547.
The synthesis and properties of a malarial GPI prototype and one variant are reported in this paper.

Exhibit D

Gas chromatogram of 4-galactosyl-xylose



Retention Time (min)	Peak Height (uV)	Area (.1*uV*sec)	Area % (%)	Plates (N)
18.700	138499	46240450	92.566	36622
18.928	33256	3266899	6.540	123711
19.713	4427	406455	0.814	104794
21.075	362	40018	0.080	81899
	192093	49953820		